

Original Research Article

Phytochemical Screening and Antimicrobial Activity of Four Members of Family Apiaceae

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Abstract

The aqueous extract of the four members- *Hydrocotyle javanica* Thunb., *Hydrocotyle rotundifolia* Roxb., *Eryngium foetidum* L. and *Centella asiatica* L.; of the family Apiaceae were screened for the phytochemicals and for antimicrobial activity against standard microbial strains by *in vitro* antimicrobial assay using agar well diffusion method. Phenolics, flavonoids and tannins were tested positive in all the four plant extracts. *H. javanica* emerged to be an effective inhibitor of five of the eight tested microbial strains. *B. subtilis* exhibited resistance to all the four plants. *H. rotundifolia* inhibited two strains which were less susceptible to the other plants. The study showed that these traditionally used herbs can be a useful source of antimicrobial agents and their use in the traditional system is scientifically justified.

Keywords: Apiaceae, Extractive value, Phytochemical screening, Antimicrobial activity, Activity Index

Introduction

Plants have been a source of medicine and a major resource for health care since ancient times, with some traditional herbal medicines having been in use since prehistoric times. Plant based medicines have been man's prime therapeutic weapons to rescue him from the clutches of diseases [1]. Recently, plant derived substances have gained popularity and have become of great importance owing to their versatile applications[2] and biocompatibility. Currently, the modern pharmaceutical industry is paying attention to plants as scientists re-discover that plants are an almost infinite resource for medicine development. It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active compounds in use today have been discovered from plant derived components following upon the ethno medicinal use of the plants[3]. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. The search and use of drugs and dietary supplements derived from plants have been accelerated in recent years. Ethnopharmacologists, botanists, microbiologists and natural product chemists are combing in their efforts for exploring medicinal flora for biological substances that could be used as such or developed for the treatment of infectious diseases. The present study is a contributing work towards evaluating the traditional knowledge of medicinal plants. The objective of this study was to screen the antimicrobial potentiality of ethno-medicinally used herbs of the Apiaceae family in order to search for

potent candidate(s) for obtaining bioactive antimicrobial compounds.

The family Apiaceae has a rich history of traditional use. The family, mostly of herbs, can be found in northern temperate regions and in tropical highlands located throughout the world. It is one of the largest families of flowering plants, having around 300 genera and 2,500 to 3,000 species. The family is defined by its distinctive umbrella-like inflorescence, the umbel, from which its alternate name- Umbelliferae, is derived. Many plants in this family have estrogenic properties and have been used as a folk medicine for birth control, as sedative and in treatments for arthritis, jaundice, asthma, as diuretics, carminative, antispasmodics and as stimulants for uterine contraction, in the treatment of diarrhoea; halitosis and genitourinary problems [4].

Materials and Methods

Chemicals

Microbiological media- Nutrient broth and agar, Malt yeast broth and agar were obtained from HiMedia, Mumbai, India. Ciprofloxacin, Clotrimazole and other general purpose laboratory chemicals and reagents were procured from Merck Specialities Pvt. Ltd, Mumbai, India.

Plant material

Plants of four members of the family Apiaceae- *Hydrocotyle javanica* Thunb., *Hydrocotyle rotundifolia* Roxb., *Eryngium foetidum* L. and *Centella asiatica* L.; were collected randomly from their natural habitats in Dibrugarh University campus. The collected species were authenticated in the Department of Life Sciences, Dibrugarh University.

Preparation of plant extracts

Air-dried plant materials were powdered and weighted, then extracted by cold maceration for 48 hours using sterile distilled water as solvent. The mixture was filtered and then centrifuged at 3500 rpm for 20 minutes. The supernatant was filtered again through Whatman No. 1 filter paper and the following filtrate was filtered through 0.2 μm membrane filter. The extracts thus obtained were evaporated to dryness in IKA -RV 10 Digital rotatory evaporator and preserved aseptically at 4°C for further use.

Determination of extractable matter

The extractable matter was determined by macerating 4.0 g of coarsely powdered air-dried material with 100ml of sterilized distilled water for 18 hours. 25 ml of the filtrate was transferred to a tared flat-bottomed dish and allowed to evaporate to dryness on a water-bath followed by drying at 105 C for 6 hours. It was allowed to cool in a desiccator for 30 minutes and then weighed immediately. The content of extractable matter was reported as extractive value and was calculated as mg per g of air-dried plant material (mg/g ADPM)[5].

Qualitative Phytochemical Screening

The aqueous extracts were subjected to phytochemical tests for detecting plant secondary metabolites- alkaloids, flavonoids, saponins, sterols and tannins. The analyses were performed by standard procedures[6][7][8].

Microorganisms and media

Seven bacteria and one fungus were selected based on literature review and their frequent interactions with human host. These were obtained from MTCC, IMTECH, Chandigarh, India. Gram positive bacteria included-*Bacillus subtilis* MTCC 441, *Staphylococcus epidermidis* MTCC 435 and *Bacillus cereus* MTCC 430. Gram negative bacteria included-*Proteus mirabilis* MTCC 1429, *Escherichia coli* MTCC 739, *Salmonella enterica* MTCC 3219, *Pseudomonas aeruginosa* MTCC 1688 and fungus-*Candida albicans* MTCC 3017. The stock cultures of bacteria were maintained in nutrient broth and that of the fungus was maintained in malt yeast broth.

Preparation of inoculum

The inoculum were prepared by diluting the stock cultures using nutrient broth for bacteria and malt yeast broth for fungi, to obtain optical density equal to that of 0.5 McFarland standard which corresponded to a cell density of 10^6 CFU mL⁻¹.

Determination of antimicrobial activity

The antibacterial activity of the extracts was determined by the agar-well diffusion method [9][10]. A known amount of each extract

was dissolved in DMSO to obtain a concentration of 200 mg/ml. One hundred microliters of the standardized inoculum of bacteria and fungi was spread on nutrient agar for antibacterial activity experiment and on malt yeast agar for antifungal activity respectively. Wells were then bored into the agar using a sterile 6 mm diameter glass well borer. Approximately 100 μL of the crude extract at 200 mg/mL was introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C for 24 hours. The diameter of zone of inhibition was measured using a transparent ruler. The experiment was performed in triplicate and the mean of diameter of zone of inhibition was calculated. The antimicrobial activity of the extracts was compared with the standard drugs- Ciprofloxacin (10 $\mu\text{g}/\text{ml}$) for bacteria and Clotrimazole (30 $\mu\text{g}/\text{ml}$) for fungi. DMSO was used as negative control.

Determination of activity index

The activity index (AI) of an extract was defined as the ratio of the mean of zone of inhibition of the extract to that of the standard drug and was calculated using the following equation[11]-

$$AI = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Mean of zone of inhibition of standard antibiotic drug}}$$

Results and Discussions

The four plants- *Hydrocotyle javanica*, *Hydrocotyle rotundifolia*, *Eryngium foetidum* and *Centella asiatica* have been used traditionally in cuisine in the form of freshly prepared aqueous pastes, popularly known as 'chutneys'. These plants have a long history of use in treatment of various human diseases and therefore were the focus of the current study. Water was selected as the solvent for extraction because the traditional preparations are prepared in aqueous medium. The water soluble extractable matter is reported in Figure 1.

A statistically significant difference (P = 0.001) was observed among all the extracts. Water-soluble extractable matter was highest for *C. asiatica* (479.00 ± 2.64 mg/g ADPM) followed by *H. rotundifolia* (419.00 ± 2.64 mg/g ADPM), *H. javanica* (370.66 ± 3.05 mg/g ADPM) and *E. foetidum* (330.33 ± 4.50 mg/g ADPM). Extractive values indicate the amount of active constituents extracted with a solvent from a given amount of plant material[5]. Among the studied plants, the higher extractive values were observed for *C. asiatica* and *H. rotundifolia* as compared to *H. javanica* and *E. foetidum*. The difference in the extractive values might be caused by the differences in the type and/or quantity of the active components. Consistency in the extractive values may be checked for quality control purposes during extract preparation. The results of qualitative phytochemical screening of the aqueous extracts of the plants are summarized in Table 1.

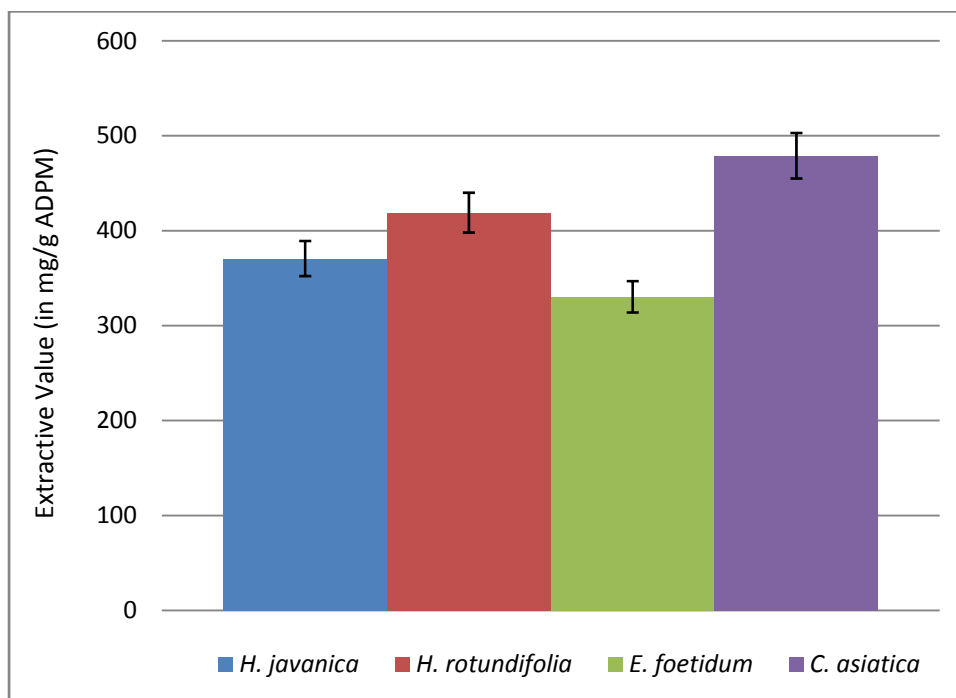


Figure 1: Water-soluble Extractable matter of the four plants

Table 1: Phytochemical screening of aqueous extracts

Aqueous extract	Phytochemicals				
	Alkaloids	Flavonoids	Saponins	Sterols	Phenolics and Tannins
<i>H. javanica</i>	-	+	+	-	+
<i>H. rotundifolia</i>	-	+	-	+	+
<i>E. foetidum</i>	-	+	+	-	+
<i>C. asiatica</i>	-	+	-	-	+

Flavonoids, phenolics and tannins were recorded in all the plants, while alkaloids were absent in all. *H. javanica* and *E. foetidum* contained saponins in addition to flavonoids, phenolics and tannins but *H. rotundifolia* recorded sterols instead of saponins. Phytochemicals are reported to exert a wide array of biological activities in plants [12][1]. Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. They belong to the polyphenol family and have several proven medicinal properties [13]. Flavonoids are synthesized by plants in response to microbial infections and this is evident in *in vitro* antimicrobial activity assays. Tannins describe a group of polymeric phenolic substances. Tannins are reported to exert diverse human physiological activities including anti-infective actions [14]. The members of the Genus *Eryngium* have been reported to contain saponins as triterpenoid derivatives as polyhydroxylatedoleanenitriterpenoids [15]. In the family Apiaceae, *Hydrocotyle* genus has also been reported to contain

polyhydroxylated triterpenoid saponins [16]. Our findings are in agreement with these reports. The aerial plant parts of *E. foetidum* are known to exhibit selective antibacterial activity against *Salmonella* species [17]. Our study showed similar results for the aqueous extract of *E. foetidum*. In addition, inhibition of both gram positive and gram negative bacteria and fungus was observed for the aqueous extract of *E. foetidum* (Figure 2 & 3).

The strains exhibited differential susceptibility towards the extracts with *B. subtilis* showing resistance to all the extracts at the tested concentration (Figure 2). The negative control did not inhibit any of the test strains. Activity Index (AI) compares the inhibition of test strains by the plant extract to that by the standard drug. Among the studied plants, *H. javanica* showed highest inhibitory activity against five bacteria- *B. cereus* (AI= 0.63), *E. coli* (AI= 0.45), *S. enterica* (AI= 0.44), *P. aeruginosa* (AI= 0.53), *S. epidermidis* (AI= 0.37), of which two are gram positive and three gram negative.

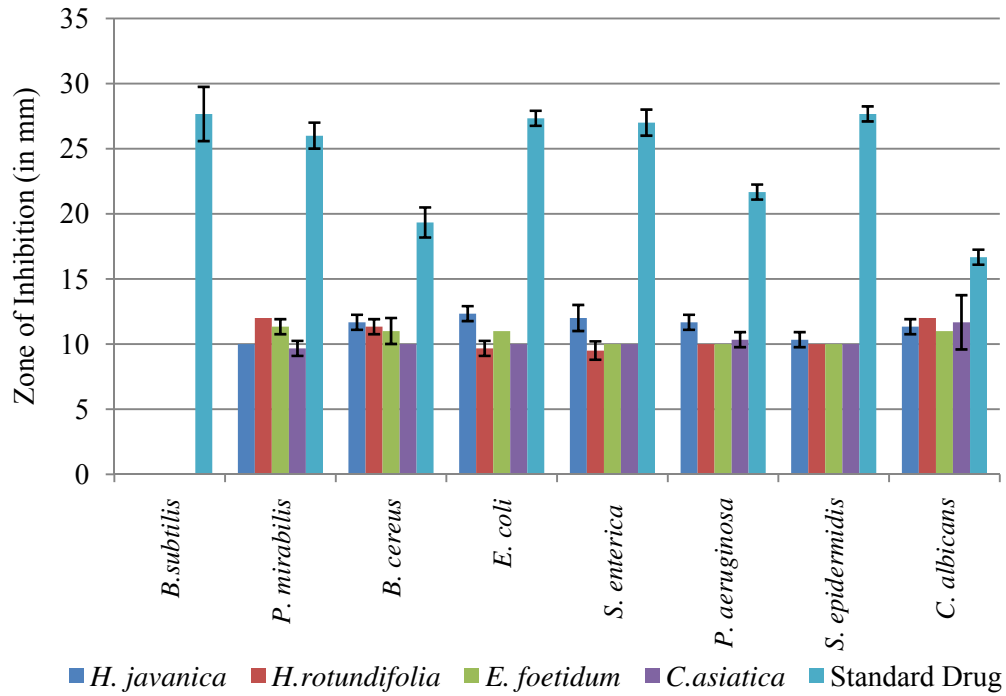


Figure 2: Antimicrobial activity of aqueous extracts.

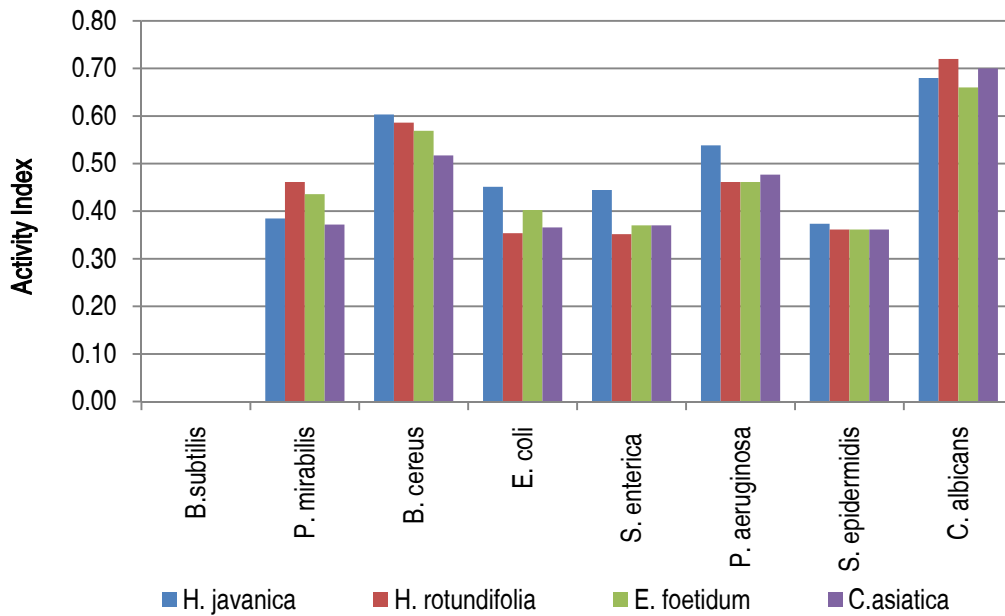


Figure 3: Activity Index of aqueous extracts



This establishes the broad spectrum effectiveness in inhibiting common human commensal which are also opportunistic pathogens. *P. mirabilis*, which is a common flora of the gastrointestinal (GI) tract and responsible for GI tract infections, was effectively inhibited by *H. rotundifolia*. *H. rotundifolia* also proved effective against in inhibiting *C. albicans*, which is commonly present in mouth and gut of healthy individuals, but causes skin and urinary infections when the growth remains unchecked.

Conclusions

The present work evaluated and established the antimicrobial activity of the four herbs commonly used in traditional cuisine and medicine. The results suggest that the format in which these herbs are used in traditional preparations does possess health benefits. The present study provides lead for pursuing further studies towards evaluation of antimicrobial activity in other solvents and for the isolation of the bioactive components.

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